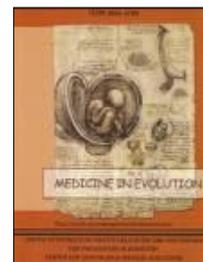# MULTIPLE ELECTRODES AGGREGOMETRY - A NEW METHOD OF ASSESSMENT OF ASPIRIN LOW RESPONDER STATUS IN PATIENTS UNDERGOING PERCUTANEOUS CORONARY INTERVENTION

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ABSTRACT

Background and objectives: Recent studies showed that clopidogrel "low- responders" patients with non-ST segment elevation acute coronary syndrome (NSTE ACS) are at high risk for development of new ischemic events. The assessment of "low- or non-responder" status to dual antiplatelet therapy (aspirin and clopidogrel) in patients undergoing percutaneous coronary intervention (PCI) with coronary stent placement for NSTE ACS may be a useful method in order to detect the high-risk patients for future cardiovascular (CV) ischemic events.

Methods: We prospectively studied the platelet response to both aspirin and clopidogrel in 100 NSTE ACS consecutive patients undergoing PCI with stenting for NSTE ACS admitted in Timisoara Institute of Cardiovascular Diseases CathLab during the year 2010. A single blood sample was obtained just before PCI and analyzed by platelet aggregometry using both arachidonic acids (AA) and ADP as agonists to detect the responder or non-responder status to aspirin and clopidogrel, respectively. We used the multiple electrode aggregometry (MEA) performed with Multiplate® analyzer by Dynabyte, Munich, Germany in order to assess the post- treatment platelet reactivity. Patients were stratified into quartiles according to the AA- and ADP - induced platelet aggregation. Patients of the highest quartile (quartile 4) were defined as the "low-responders".

Results: Ten recurrent cardiovascular (CV) events occurred during the 1- month follow-up. Clinical cardiovascular events were significantly associated with platelet response to clopidogrel [Quartile 4 vs.1, 2, 3: OR (95% CI) 19.8 (4.4-90.8; P<0.0001)].

Conclusions: The MEA assessment of AA- and ADP- induced platelet aggregation is a valuable method to identify the low- responders to dual antiplatelet therapy. The results correlated the values of area under curve (AUC) determined by MEA with the aspirin and clopidogrel low- responder status. The clopidogrel low- responders (quartile 4) were identified as the category of patients at high risk for recurrent cardiovascular ischemic events.

Key words: aspirin, clopidogrel, low- responder, multiple electrodes aggregation

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INTRODUCTION

The concept of "aspirin and clopidogrel resistance" still represents a provocative theme in the literature [1-4]. So far, there are a lot of studies published in this area, but its definition, diagnosis, etiology and clinical implications remain uncertain [1, 5].

Definition of antiplatelet response variability or "resistance"

The term of "resistance" to a drug should be used when the drug is unable to hit its pharmacological target, due to inability to reach it (consequence of different factors: reduced bioavailability, negative interaction with other drugs, *in vivo* inactivation) or to alteration of the target [1, 5].

Accordingly to this definition, the term of resistance to aspirin should be limited to situations in which aspirin is unable to inhibit COX-1-dependent thromboxane A_2 (Tx A_2) production and, consequently, TxA_2-dependent platelet functions [5].

Platelet activation is a complex process, comprising multiple signaling pathways that mediate and initiate thrombotic events. In consequence, a treatment strategy directed against a single pathway cannot be expected to prevent the occurrence of all events [6]. Therefore, treatment failure alone is not sufficient evidence of drug "resistance".

The optimal definition of "resistance" or nonresponsiveness to an antiplatelet agent might be evidence of persistent activity of the specific target of the antiplatelet drug [7, 8].

MATERIAL AND METHOD

Study patients

The study patients were consecutively admitted to the Institute of Cardiovascular Diseases Timisoara during the year 2010. Percutaneous coronary intervention (PCI) with stent implantation was performed in the CathLab of the Institute of Cardiovascular Diseases Timisoara in all study patients.

They were eligible for this prospective study if they had presented clinical symptoms defined as acute myocardial ischemia within 12 hours before admission and at least one of the following criteria: transient (< 20 min) ST-segment elevation > 0.1 mV, a new finding of ST-segment depression >0.05 mV, T-wave inversion in at least two contigues leads, increased level of cardiac ischemic biomarkers or coronary disease documented by a previous coronary angiography, coronary revascularization or myocardial infarction. The exclusion criteria were: ST elevation ACS, NYHA class IV, PCI or coronary artery by-pass grafting (CABG) in the last 3 months, use of antiGPIIb/IIIa therapy before the PCI procedure, history of bleeding diathesis, contraindication to antiplatelet therapy, platelet count < 100 $10e^6L^{-1}$, creatinine clearance < 30 ml/min.

Patients on chronic clopidogrel therapy with a daily dose of 75 mg > 5 days did not received a loading dose of clopidogrel. Other patients received a loading dose of 300 mg clopidogrel at least 12 hours before the PCI. All patients received aspirin doses (75- 300 mg) daily administered at least 12 hours before stenting procedure.

The study protocol was approved by the Ethics Committee of the Institute of Cardiovascular Diseases Timisoara, and patients gave informed consent for

participation. PCI was performed within 48-72 hours after hospital admission.

Blood samples

Blood samples for testing the platelet activity by multiple electrodes aggregometry method were drawn after admission in the CathLab, before PCI at least 12 h after the loading dose of the clopidogrel and aspirin administration and before administration of anti GP IIb/IIIa therapy if needed. The blood was collected in special vacutainer tube provided by the Multiplate® analyzer manufacturer containing usually hirudin, filled to capacity, and then inverted three to five times for gentle mixing before the laboratory analysis testing.

Laboratory method

The aggregation testing was performed in Timisoara Institute of Cardiovascular Diseases Laboratory. We used the multiple electrodes aggregometry method performed with the Multiplate® analyzer, produced by manufacturer Dynabyte Medical, Munich, Germany. Multiplate® reagents are also provided by the manufacturer, as reconstitute solutions called ASPItest and ADPtest. In all patients we determined inhibition of arachidonic-acid induced aggregation (ASPItest), which is in accordance with an adequate aspirin effect and inhibition of ADP induced aggregation (ADPtest) which is in accordance with an adequate clopidogrel effect.

The method is a fast and easy one, comprising a few specific steps and allows obtaining the printed result in a short time (10 minutes). The result is represented by a graphic curve of aggregation (AU) /time (minutes). The parameters of the results are: the velocity (AU/min), the aggregation (AU), and the most important is area under the curve AUC expressed in AU*min or U (10 AU*min= 1U).

Clinical endpoint

The clinical endpoint included all following major cardiovascular events (MACE): cardiovascular (CV) death, acute or subacute stent thrombosis, recurrent ACS and ischemic stroke. Follow-up events were prospectively assessed by routine clinical 30 days follow-up after PCI (15). ACS was defined by the presence of symptoms compatible with recurrent ischemia needing new hospitalization and angiocoronarography, ischemic stroke was defined as a new focal neurological deficit without bleeding on computer tomodensitometry (CT) and confirmed by a neurologist.

Statistical analysis

Statistical analysis was performed with the SAS Software (SPSS Inc., Chicago, IL, USA). Continuous variables are expressed as mean± SD. Categorical variables are expressed as frequencies and percentages. The Wilcoxon rank- sum test was used to compare continuous variables in individuals with and without CV events. We used the Fischer's exact test when frequencies were below five and the χ^2-test to compare the categorical variables. *P* for trend between quartiles of AUC values of AA-or ADP-induced aggregation and other variables was studied using a general linear model with AA- or ADP- induced aggregation as dependent variable. Comparison between individuals with maximal intensity of AA- or ADP- induced aggregation in the top vs. the three bottom quartiles were performed using logistic regression after adjustment for conventional CV risk factors, treatment and inflammatory parameters. ADP and AA-induced maximal intensity of pla-

telet aggregation expressed as AUC value/patient were analyzes as potential predictors of the clinical endpoint both univariably and after adjustment for others baseline confounding variables.

The value of P<0.05 was considered significant.

RESULTS

Patients' characteristics

One-hundred patients were included in our prospective study. Twenty-one patients (20%) were on chronic clopidogrel therapy. Demographic and biological baseline data of the studied population are summarized in Table1. The mean age was 63.2± 12 years, and 77% of the patients were males. Multiple CV risk factors were frequent (27% of patients were with diabetes mellitus, 57% were hypertensive, 64% presented dyslipidemia). For the patients who did not received clopidogrel prior to the current admission, the mean time between the clopidogrel loading dose and blood sampling was 16± 2.5 hours. All patients received a daily 75 mg clopidogrel dose and a daily aspirin dose of 100 mg during the 1 month follow-up period. The mean time between angina symptoms and PCI was similar in patients with or without recurrent ischemic CV events (mean± SD = 20±4.63 vs. 20.24±3.97, P= 0.78).

Platelet response to aspirin and clopidogrel

We analyzed platelet response to aspirin and clopidogrel using the multiple electrodes agreggometry performed with Multiplate® analyzer. In all patients we determined inhibition of AA- (ASPItest) and ADP- induced aggregation (ADPtest). The result of the test is represented by a curve of aggregation (AU) /time (minutes). The most important parameter is area under the curve AUC expressed in AU*min or U (10 AU*min= 1U), which is in accordance with an adequate aspirin and clopidogrel effect, respectively. Other important parameters assessed in the study population were the velocity (AU/min) and the aggregation (AU).

We observed that the distribution of the AUC values representing the AA- induced platelet aggregation was not consistent with a normal distribution and permitted to identify 3 groups of patients with no, intermediate and high response to aspirin. ADP- induced platelet response intensity was consistent with a normal, bell-shaped distribution.

According to their AA- induced platelet aggregation, patients were stratified into four quartiles based on their AA- induced platelet aggregation response, evaluated by AUC values obtained using ASPItest on Multiplate analyzer: patients into the first quartile (Q1)- had AUC ASPItest < 400 AU*min, the second quartile (Q2)- patients presented AUC ASPItest values between 400 and 600 AU*min, the third quartile (Q3)- patients, AUC ASPItest in the 600-700 AU*min interval, and the fourth quartile-patients with AUC ASPItest values over 700 AU* min. The mean AUC values resulted on ASPItest were respectively 326.3± 108.2 AU*min, 534 ± 58.2 AU*min, 667±23.4 AU*min, 902±125.8 AU*min from the first to the fourth quartiles.

We classified also the entire study group into four quartiles according to their ADP-induced platelet aggregation response, assessed by the AUC values,

respectively. We considered the first quartile (Q1) of patients " clopidogrel high-responders" (AUC < 200 AU* min), the second quartile of patients (Q2)- "clopidogrel responders" (200 AU*min <AUC < 500 AU*min), the third quartile (Q3)- "clopidogrel intermediate responders" (500 AU*min <AUC< 700 AU*min) and the patients from the fourth quartile (Q4) were "clopidogrel low responders" (AUC >700 AU*min).

Table 1 Baseline characteristics of the patients with and without cardiovascular events

Characteristics	Group without CV events (n= 90)	Group with CV events (n=10)	P value
Age, years	61 ± 8	64 ± 4	0.84
Male,n (%)	70 (77)	8 (80)	0.62
Current smoker, n (%)	50 (55)	9 (90)	0.48
History of ACS, n (%)	47 (52)	6 (60)	0.49
Diabetes mellitus, n (%)	25 (27)	3 (30)	0.22
BMI (kgxm^{-1})	24± 0.5	26 ± 1	0.42
Hypertension, n (%)	52 (57)	6 (60)	0.53
Dyslipidemia, n (%)	58 (64)	7 (70)	0.79
Troponin positive patients, n (%)	25 (27)	7 (70)	0.24
ST-segment shift, n (%)	26 (28)	3 (33)	0.79
Number of stents, n (%)	19 (21)	5 (50)	0.071
Medication			
Statins, n (%)	54 (60)	7 (70)	0.58
Beta-blockers, n (%)	65 (72)	8 (80)	0.85
IECA, n (%)	48 (53)	4 (40)	0.63
Biological parameters			
hs-CRP (mg/L) Platelet	1.1±0.5	2.8 ± 0.9	0.58
count (10^6 e/L)	224±15	247±29	0.84
Creatinin (mg /dl)	0.7±0.1	0.8±0.02	0.96
AUC (AU*min) (ADP test, MEA)	444.5±361.3	812±172.5	< 0.001

The mean ADP- induced intensity of platelet aggregation response (represented by mean AUC value done by ADPtest) in the described quartiles was: 155.5 ± 22.6 AU*min (Q1), 386.7 ± 86.13 AU*min (Q2), 599.3 ± 61.4 AU*min (Q3) and 819 ± 49.1 AU*min (Q4). The range of ADP-induced intensity of platelet aggregation (AUC) in the fourth quartile (low-responders) was 701-940 AU*min determining a cuttof value of 700 AU*min; this value is also present in a few number of stu-dies available so far regarding this new method- multiple electrodes aggregometry. There were no significant difference between the quartile one and four, exception of a significant increase of intensity of AA-induced platelet aggregation (AUC) (P for trend <0.01). No significant relation was found between ADP-induced platelet aggregation and cardiac biomarkers (the percent of troponin positive patients *P* =1.1), the presence of ST-shift (*P*=0.42) or the number of stents (*P* = 0.2).

Clinical outcomes

All the study patients were followed at 1month post PCI. Recurrences in ischemic events were evaluated as follows: ten patients (10%) presented angina episodes; one patient was hospitallized for recurrent ACS because of an acute stent thrombosis, one CV death and no ischemic stroke. Patients which presented CV events at 1- month follow-up had a significantly increased ADP-induced platelet aggregation (P < 0.0001).

A gradual increasing number of CV events were observed from the first to the fourth quartile (0, 0, 2, and 8 respectively).

In addition, quartile 4 was associated with a higher risk of CV events as compared with the others: the odds ratio (OR) associated with the top versus lower quartiles was 19.8 (95% CI = 4.4-90.8), P<0.001 and this difference remains significant after the adjustment for age, sex, CV factors, and treatment- OR 35 (95% CI = 4.85-246), P<0.001.

DISCUSSION

Aspirin "Resistance"

Aspirin acetylates a serine moiety present in cyclooxygenase-1 (COX-1). It irreversibley inhibits the COX-1- dependent synthesis of thromboxane A_2 (TxA$_2$), which is essential for the full aggregation response of platelets [9]. Aspirin resistance is infrequent among patients undergoing elective PCI who are treated with 325 mg daily as assessed

by arachidonic acid-induced platelet aggregation with LTA [10, 11].

The incidence of aspirin resistance seems to be highly assay-dependent and is rare when determined by methods that directly indicate the activity of COX-1 [12, 13].

Treatment non-compliance can also affect the identification of aspirin "resistance" [10, 11].

Table 2 Laboratory assessment of platelet responsiveness to aspirin

COX-1 specific methods	COX-1 Non-Specific methods

1.AA- Induced Platelet Aggregation
  -LTA (PRP,whole blood)
  -TEG (whole blood)
  -VerifyNow (whole blood)
  - Multiplate
	1.ADP and Collagen-Induced Platelet Aggregation
	  -LTA

2.Thromboxane Metabolite
  - Serum
  - Urine
	2. Shear, Collagen/epinephrine-induced Platelet Aggregation
	  -PFA-100

Aspirin resistance also might to be associated with concomitant clopidogrel "resistance"[13, 14]. Patient's identified as aspirin-and clopidogrel-resistant has exhibited high platelet reactivity to collagen in addition toADP and arachidonic acid stimulation [13-15]. Re-

cent studies suggest a generalized high-platelet-reactivity phenotype that might be associated with an increased risk for ischemic events.

Laboratory assessment of platelet responsiveness to aspirin can be divided in COX-1 –specific and COX-1-

nonspecific. Arachidonic acid (AA) stimulation of platelet aggregation depends directly on COX-1 activity. In vivo production of TxA_2 is assessed by measurement of stable metabolites via enzyme-linked immunoassays. Adenosine diphosphate (ADP) - and collagen-stimulated aggregation are COX-1-nonspecific methods. Aggregation occurs through COX-1-independent and-dependent pathways after stimulation using latter agonists [6] (Table 2).

Table 3 Laboratory assessment of Clopidogrel responsiveness

Receptor reactivity- intracellular signaling downstream from the $P2Y_{12}$	- Flow cytometry - VASP
Receptor expression	- Flow cytometry - P-selectin
Aggregation	- LTA - MEA - VerifyNow - Thrombelastography

Clopidogrel "Resistance"

The active metabolite of Clopidogrel is responsive of irreversibly inhibition of $P2Y_{12}$ receptor, because of the new covalent disulfide bond; in cases of non-responder patients there are evidence of posttreatment $P2Y_{12}$ reactivity. For aspirin, the diagnosis of resistance would use a laboratory technique that detects residual activity of cyclooxigenase COX-1. The proposed mechanisms of antiplatelet response variability /resistance were described in a large number of publications [1-5, 7].

The status of "clopidogrel non-responder" was mainly detected using LTA in a large number of studies and using ADP as an agonist [6, 8-24, 26, 36]. Also different methods were used to identify the clopidogrel nonresponsiveness: point-of-care assays, VASP phosphorylation, flow-cytometric measurement of activation-dependent receptor expression after ADP stimulation [16, 17-20, 21-25].

The therapeutic response to clopidogrel has been most studied in patients undergoing PCI, and numerous studies have reported wide variations in response to therapy and rates of nonresponders of 5% to 44% [6-19]. Determination of VASP phosphorylation has also shown high residual reactivity of the $P2Y_{12}$ receptor in selected patients treated with clopidogrel [26]. Differences in the prevalence of non-responder status in different studies might be related to differences in definitions (relative versus absolute change in aggregation, maximum versus late aggregation), laboratory methods, different dosages. Clopidogrel response variability has multiple proposed etiologies.

Laboratory assessment of Clopidogrel responsiveness comprises different methods. ADP stimulates distinct receptors ($P2Y_1$ and $P2Y_{12}$) that are linked to specific signaling pathways.

Response can be measured by:

1. Receptor reactivity; intracellular signaling downstream from the

P2Y$_{12}$ receptor is measured by flow cytometry that assesses phosphory-lation of vasodilator- stimulated phosphoprotein (VASP) with monoclonal antibodies; the P2Y$_{12}$ is coupled by a Gi protein to adenylyl cyclase which activates protein kinaseA (PKA).

2. Activation–dependent receptor expression (active glycoprotein GP IIb/IIIa and P-selectin) identified by monoclonal antibodies (Y) with flow cytometry.

3. Aggregation determined by light transmittance aggregometry (LTA), multiple electrodes aggregometry (MEA- whole blood platelet aggregation assessed with the Multiplate analyzer), aggregation of platelets with fibrinogen-coated beads (VerifiNow), or measuring the contribution of platelet aggregation to total platelet fibrin clot strength by thrombelastography (Table 3).

Methods used to measure platelet function during antiplatelet treatment

Light transmittance aggregometry (turbidimetric method, LTA) has been the most widely used technique to monitor the effect of antiplatelet drugs, including aspirin, clopidogrel, other P2Y12 inhibitors, and platelet glycolprotein (GP) IIb/IIIa inhibitors [26, 27]. In the studies using LTA, the historical "gold standard" test, based on the stimulation of platelet-platelet aggregation in plateletrich plasma after stimulition with various agonists, we identified disadvantages related to the laboratory employees' workload. Potential disadvantages include the immediate processing, variable reproducibility, large required sample volumes, lengthy processing time, and expenses of the aggregometer and trained operators. LTA has also been the most widely investigated method to predict clinical outcomes [28]. Impedance aggregometry

is conceptually similar to LTA, but it uses whole blood instead of platelet-rich plasma and platelet aggregation is measured by impedance, not by light transmittance [29].

Platelet function analysis using multiple electrode aggregometry (Multiplate®)-Dynabyte, Munich, Germany is a recent method which allow an easy and fast assessment of platelet function, with the possibility to decide on treatment regimens when the patient is still in the CathLab (results in 10 minutes). In present, Multiplate is used in many expert centers and pharmaceutical companies throughout Europe. This method is also suitable for daily clinical practice for many reasons- were eliminated time consuming etapes related to rich platelet plasma preparation or light transmittance aggregometer manipulation. This new method of aggregometry allow a rapid and reliable results, could be used also in the Coronary Unit or CathLab. So far, a large number of studies were focused on individual variability of platelet response to clopidogrel and the term of "clopidogrel resistance" is usual [20-26]. Despite these facts, its definition is still controversial and mainly based on the percentage change in ADP-induced maximal intensity of platelet aggregation before and after initiation of clopidogrel treatment (clopidogrel responsiveness). In addition, the cut-off value to identify the low responders varied in large ranges (from <10% to 40%) [20-26].

Our study demonstrated that, among a high risk category of CV patients, admitted for NSTE ACS treated by PCI with stenting, the single ADP-induced of platelet aggregation measurement using multiple electrodes aggregometry (MEA) was associated with the subsequent occurrence of major adverse CV events (MACE).

In our study, a baseline aggregation assessment could not be obtained beca-

use of certain factors: previous chronic clopidogrel therapy, patients' admission through the Emergency Department.

But recent studies demonstrated that pretreatment platelet activity did not predict the clopidogrel responsiveness [25, 14]. These trials have also shown the correlation between a low response to clopidogrel (difference between pre- and posttreatment values) and high post- treatment platelet activity which was proposed as a better estimate of thrombotic risk [9, 14, 16-19]. Therefore we performed one test per patient to assess the clopidogrel effect with ADP-induced platelet aggregation, from one single blood sample just before PCI, without baseline determination.

Patients were stratified into quartiles according to their post therapy ADP induced platelet aggregation represented by the AUC value obtained with the ADPtest using multiple electrode aggregometry performed with the Multiplate® analyzer. The patients of the fourth quartile were characterized as "clopidogrel low responders" (AUC >700 AU*min). In addition, we considered the first quartile (Q1) of patients "clopidogrel high-responders" (AUC < 200 AU*min), the second quartile of patients (Q2) - "clopidogrel responders" (200 AU*min <AUC < 500 AU*min), the third quartile (Q3) - "clopidogrel intermediate responders" (500 AU*min <AUC< 700 AU*min). Simi-

larly, in other studies the patients from the fourth quartile were defined as the low responders and were characterized by a maximal intensity of ADP-induced platelet aggregation >70% Gurbel et al [16], Cuisset et al [20]. A relation between clopidogrel resistance and recurrence of clinical CV ischemic events is emerging. Correlation of ADP induced platelet aggregation with clinical outcomes was showed for the first time in ST elevation ACS by Matetsky et al [14]. These data and a lot of results from clinical trials strongly suggested that the clopidogrel resistance might be associated with an increased risk of recurrent CV events [21-28].

In this study, in the 1- month post PCI follow-up period, a number of 10 CV ischemic events occurred. We demonstrated a correlation between clopidogrel response defined on a single blood sample before the PCI and recurrence of CV ischemic events for NSTE ACS patients undergoing coronary stenting; in addition, accordingly with the quartile stratification, a number of 8 events (80%) occurred in the 4-th quartile patients, characterized as "clopidogrel low-responders", and only 2 events (20%) in the third quartile patients- "clopidogrel intermediate responders". No CV events were registered to the patients from the first and second quartile, characterized as "clopidogrel high-responders" and "clopidogrel responders".

CONCLUSION

The results of the present study are encouraging. We found out a correlation between clopidogrel platelet response and a subgroup of patients at higher risk of recurrent ischemic CV events after stenting for NSTE ACS. In addition, we used a single blood sam-

ple per patient for testing post- treatment AA- and ADP-induced platelet aggregation and a new, faster, simple and accurate method to assess the platelet aggregation. The cutt-of values of AUC using multiple electrodes aggregation (MEA) were useful to identify

the "aspirin and clopidogrel low-responders."

Perspectives – MEA seems to be a valuable method to identify the aspirin and clopidogrel low responders. This high- risk patients may potentially benefit from a more aggressive antithrombotic therapy (higher clopidogrel doses, alternative molecules, combined antiplatelet therapy) [29].

REFERENCES

1. Cattaneo M. Aspirin and Clopidogrel. Efficacy, safety and the issue of drug resistance. Arterioscler Thromb Vasc Biol 2002; 24:1980-1987.

2. Patrono C. Aspirin resistance: definition, mechanisms and clinical read-outs. J Thromb Haemost 2003; 1:1710-1713.

3. Campbell CL, Steinhubl SR. Variability in response to aspirin: do we understand the clinical relevance? J Thromb Haemost 2005; 3: 665-669.

4. Undas A, Brummel-Ziedins KE, Mann KG. Antithrombotic properties of aspirin and resistance to aspirin: beyond strictly antiplatelet actions. Blood 2007; 109: 2285-2292.

5. Cattaneo M. Laboratory detection of 'aspirin resistance': what test should be use (if any)? Eur Heart J 2007; 28: 1673-1675.

6. Gurbel PA, Becker CR, Kenneth GM, Steinhubl SR, Michelson AD. Platelet function monitoring in patients with coronary artery disease. J Am Coll Cardiol 2007; 50: 1901-20.

7. Gurbel PA, Tantry US. Clopidogrel resistance? Thromb Res 2007; 120:311-321.

8. Gurbel PA, Becker CR, Kenneth GM, Steinhubl SR, Michelson AD. Platelet function monitoring in patients with coronary artery disease. J Am Coll Cardiol 2007; 50: 1901-20.

9. Michelson AD. Platelet function testing in cardiovascular diseases. Circulation 2004; 110:e489-e493

10. Tantry US, Bliden KP, Gurbel PA. Overestimation of platelet aspirin resistance detection by thrombelastograph platelet mapping and validation by conventional aggregometry using arachidonic acid stimulation. J Am Coll Cardiol 2005; 46: 1705-1709.

11. Schwartz KA, Schwartz DE, Ghosheh K, Reeves MJ, Barber K, DeFranco A. Compliance as a critical consideration in patients who appear to be resistant to aspirin after healing of myocardial infarction. Am J Cardiol 2005; 95: 973-975.

12. Gurbel PA, Bliden KP, Di Chiara J, et al. Evaluation of dose- related effects of aspirin on platelet function: results from the Aspirin- Induced Platelet Effect (ASPECT) study. Circulation 2007; 115:3156-3164.

13. Pulcinelli FM, Riondino S, Celestini A, et al. Persistent production of platelet thromboxane A_2 in patients chronically treated with aspirin J Thromb Haemost 2007; 3: 2784-2789.

14. Templin C, Schaefer A, Stumme B, Drexler H, von Depka M. Combined aspirin and clopidogrel resistances associated with recurrent coronary stent thrombosis. Clin Res Cardiol 2006; 95: 122-126.

15. Dichiara J, Bliden KP, Tantry us, et al. Platelet aspirin responsiveness measured by VerifyNow: correlation with multiple methods and identification of a high platelet reactivity phenotype. Platelets 2007; 18: 414-483.

16. Gurbel PA, Bliden KP, Hiatt BL, O'Connor CM. Clopidogrel for coronary stenting: response variability, drug resistance, and the effect of pretreatment platelet reactivity. Circulation 2003; 107:2908-2913.

17. Geisler T, Langer H, Wydymus M,et al. Low response to clopidogrel is associated with cardiovascular outcome after coronary stent implanttation. Eur Heart J 2006; 27:2420-2425.

18. Hochholzer W, Trenk D, Bestehorn HP, et al. Impact of the degree of peri-interventional platelet inhibition after loading with clopidogrel on early clinical outcome of elective coronary stent placement. J Am Coll Cardiol 2006; 48:1742-1750.

19. Buonamici P, Marcucci R, Migliorini A, et al. Impact of platelet reactivity after clopidogrel administration on drug-eluting stent thrombosis J Am Coll Cardiol 2007; 49:2312-2317.

20. Gurbel PA, Bliden KP, Guyer K, et al. Platelet reactivity in patients and recurrent events post-stenting: results of the Prepare Post-Stenting Study. J Am Coll Cardiol 2005; 46:1820-1826.

21. Barragan P, Bouvier JL, Roquebert PO, et al. Resistance to thienopyridines: clinical detection of coronary stent thrombosis by monitoring of VASP. Catheter Cardiovasc Interv 2003; 59: 295-302.

22. Cuisset T, Frere C, Quilici J, Barbou F, Morange PE, Hovasse T, Bonnet JL, Alessi MC.J Thromb Haemost 2007; 4: 542-549.

23. Gurbel PA, Bliden KP, Samara W, et al. Clopidogrel effect on platelet reactivity in patients with stent thrombosis: results of the CREST study. J Am Coll Cardiol 2005; 46: 1827-1832.

24. Ajzenberg N, Aubry P, Huisse MG, et al. Enhanced shear- induced platelet aggregation in patients who experience subacute stent thrombosis:

a case- control study. J Am Coll Cardiol 2005; 45:1753-1756.

25. Gurbel PA, Bliden KP, Samara W, et al. Clopidogrel effect on platelet reactivity in patients with stent thrombosis: results of the CREST study. J Am Coll Cardiol 2005; 46: 1827-1832.

26. Gurbel PA, Becker CR, Kenneth GM, Steinhubl SR, Michelson AD. Platelet functionmonitoring in patients with coronary artery disease. J Am Coll Cardiol 2007; 50: 1901-20.

27. Michelson AD. Platelet function testing in cardiovascular diseases. Circulation 2004; 110: e489-e493

28. Gum PA, Kottke- Marchant K, Poggio ED, et al. Profile and prevalence of aspirin resistance in patients with cardiovascular disease. Am J Cardiol 2001; 88: 230-235.

29. Gurbel PA, Bliden KP, Hayes KM, Young S, Herzog WR, Tantry US. The relation of dosing to clopidogrel responsiveness and the incidence of high posttreatment platelet aggregation in patients undergoing coronary stenting.J Am Coll Cardiol 2005; 116: 491-7.

30. Jaremo P, Lindahl TL, Fransson SG, Richter A. Individual variations of platelet inhibition after loading doses of clopidogrel. J intern Med 2007; 252: 233-8.

31. Gurbel PA, Bliden KP, Hiatt BL, O'Connor CM. Clopidogrel for coronary stenting: response variability, drug resistance, and the effect of pretreatment platelet reactivity. Circulation 2003; 107: 2908-13.

32. Muller I, Besta F, Schulz C, Massberg S, Schonig A, Gawaz M. Prevalence of clopidogrel non- responders among patients with stable angina pectoris scheduled for elective coronary stent placement. Thromb Haemost 2008; 89: 783-7.

33. Mobley JE, Bresee SJ, Wortham DC, Craft RM, Snider CC, Carroll RC. Frequency of nonresponse antiplatelet activity of clopidogrel during pretreatment for cardiac catheterization. Am J Cardiol 2004; 93: 456-8.

34. Angiolillo DJ, Fernandez- Ortiz A, Bernardo E, Ramirez C, Barrera- Ramirez C, Sabate M, Jimenez-Quevedo P, Hernandez R, Moreno R, Escaned J, Alfonso F, Banuelos C, Costa MA, Bass TA, Pignatti PF, Macaya C. Identification of low responders to a 300- mg clopidogrel loading dose in patients undergoing coronary stenting. Thromb Res 2005; 115:101-8.

35. Serebruany VL, Steinhubl SR, Berger PB, Malinin AI, Bhatt DL, Topol EJ. Variability in platelet responsiveness to clopidogrel among 544 individuals. J Am Coll Cardiol 2005; 45: 246-51.

36. Lepantalo A, Virtanen KS, Heikkila J, Wartiovaara U, Lassila R. Limited early antiplatelet effect of 300 mg clopidogrel in patients with aspirin therapy undergoing percutaneous coronary intervention.Eur Heart J 2004; 25: 476-83.

37. Gum PA, Kottke- Marchant K, Welsh PA, White J, Topol EJ. A prospective, blinded determination of the natural history of aspirin resistance among stable patientswith cardiovascular disease. J Am Coll Cardiol 2003; 41: 961-5.

38. Davis CJ, Gurbel PA, Gttis WA, Fuzaylov SY, Nair GV, O'Connor CM, Serebruany VL. Hemostatic abnormalities in patients with congestive heart failure: diagnostic significance and clinical challenge. Int J Cardiol 2000; 75: 15-21.

39. Gurbel PA, Bliden KP, Di Chiara J, et al. Evaluation of dose- related effects of aspirin on platelet function: results from the Aspirin- Induced Platelet Effect (ASPECT) study. Circulation 2007; 115:3156-3164.

40. Cuisset T, Frere C, Quilici J, Barbou F, Morange PE, Hovasse T, Bonnet JL, Alessi MC.J Thromb Haemost 2007; 4: 542-549.

41. Vinholt P, Poulsen TS, Korsholm L, Kristensen SR, Hallas J, Damkier P, Mickley H. The antiplatelet effect of clopidogrel is not attenuated by statin treatment in stable patients with ischemic heart disease. Thromb Haemost 2005; 94: 438-43.

42. Muller I, Seyfarth M, Rudiger S, Wolf B, Pogatsa Murray G, Schomig A, Gawaz M. Effect of a high loading dose of clopidogrel on platelet function in patients undergoing coronary stent placement. Heart 2001; 85:92-3.

43. Kastrati A, von Beckerath N, Joost A, Pogatsa-Murray G, Gorchakova o, Schomig A. Loading with 600 mg clopidogrel in patients with coronary artery disease with and without chronic clopidogrel therapy. Circulation 2004; 110: 1916-9.

44. Angiolillo DJ, Fernandez- Ortiz A, Bernardo E, Ramires C, Sabate M, Banuelos C, Hernandez-Antolin R, Escaned J, Moreno R, Alfonso F, Macaya C. High clopidogrel loading dose during coronary stenting: effects on drug response and interindividual variability. Eur Heart J 2004; 25:1903-10.

45. Patti G, Colonna G, Pasceri V, Pepe LL, Montinaro A, Di Sciascio G. Randomized trial of High Loading Dose of Clopidogrel for Reduction of Periprocedural Myocardial Infarction in Patients Undergoing Coronary Intervention. Results from ARMYDA-2 (Antiplatelet therapy for Reduction of Myocardial Damage during Angioplasty) Study. Circulation 2005; 111: 2099-106.

46. Storey RF. The P2Y12 receptor as a therapeutic target in cardiovascular disease. Platelets 2001; 12: 197-209.